

n-ALKANES IN *PTEROCELASTRUS* LEAF WAX

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ABSTRACT

The n-alkanes in the leaves of three *Pterocelastrus* species are compared to each other. All specimens except one contain C_{29} as the primary alkane. One specimen of *P. rostratus* contains C_{28} as largest component. A negative correlation exists between total alkane content and the observed number of alkanes of different chain lengths. Intraspecific variation is larger than interspecific variation so that no taxonomic conclusions can be drawn from the observed patterns.

UITTREKSEL

n-ALKANE IN *PTEROCELASTRUS* BLAARWAS

Die n-alkane in die blare van drie *Pterocelastrus*-spesies word met mekaar vergelyk. Al die eksemplare behalwe een bevat C_{29} as die primêre alkaan. Een eksemplaar van *P. rostratus* bevat C_{28} as grootste komponent. 'n Negatiewe korrelasie bestaan tussen totale alkaanhoud en die waargenome aantal alkanes van verskillende kettinglengtes. Intraspesifieke variasie is groter as interspesifieke variasie sodat geen taksonomiese gevolgtrekkings uit die waargenome patrone gemaak kan word nie.

INTRODUCTION

The presence of n-alkanes as a constituent of leaf "external" (cuticular) and "internal" (cell content) waxes and their importance as a taxonomic criterion have been extensively discussed (Eglinton & Hamilton, 1967; Herbin & Robins, 1969) and applied to taxonomic problems by various authors.

Recent work has shown a possible relationship between species specific wax composition and insect susceptibility in *Trifolium* spp. (Thompson & Knight, 1978). This would seem to indicate that the wax composition, as well as the amount formed, is under genetic control and therefore could be used as a valid taxonomic attribute of a species. This is in agreement with Corrigan, Timoney & Donnelly (1978) who found that the alkane patterns of the leaves of *Galium* and *Asperula* do not change with plant age or geographical location and may be used as a taxonomic tool.

Herbin & Robins (1968) found an increase in carbon chain length with increasing age in several Gymnosperms, as was also found by Franich, Wells & Holland (1978) in *Pinus*. Similar results were obtained by Bianchi & Corbellini (1977) with leaves of *Triticum*. These authors therefore imply that the alkane composition of leaves cannot be used as a taxonomic attribute. This is also the

* This work was done at the Department of Botany, Potchefstroom University.
Accepted for publication 5th October, 1979.

conclusion to which Smith & Martin-Smith (1978) came after examination of the n-alkanes of *Saccharum* where they found that the intraspecific variation was larger than the interspecific variation.

No definite conclusions as to the suitability of this method for the examination of any taxon can thus be drawn.

During a study of *Pterocelastrus* Meisn. it was necessary to identify vegetative plant material of this genus unequivocally. Such material is more than ordinarily difficult to identify, due to the great similarity to members of other closely related genera. Proof of the difficulty of identification can be found in nearly any herbarium where non-fruiting material of especially *Maytenus oleoides* and *Pterocelastrus tricuspidatus* are mixed or misidentified. It was therefore deemed necessary to use chemotaxonomic characteristics to help with the identification.

G.L.C. determinations of the alkane composition of leaf waxes are relatively easy to perform and not unduly time-consuming. This approach was therefore chosen, especially with due regard to the equivocal results reported in the literature, so that recommendations as to the suitability of this method in *Pterocelastrus* and allied genera can be given.

Such an approach is necessary because the alkanes of any of the representatives of the Celastraceae have not been previously studied. No reference to this class of compounds can be found in the very complete treatment of the chemical compounds of the Celastraceae by Brüning & Wagner (1978).

Due to the known differences between "external" and "internal" leaf wax composition of higher plants (Herbin & Robins, 1969), and the possible differences between leaves of different ages, it was decided to analyse the total wax n-alkane component of leaves from an entire branch so that cuticular and internal alkanes of young and old leaves contribute to the pattern.

Suitable material of three species of *Pterocelastrus* could be obtained, and this was used in the analysis.

MATERIAL AND METHODS

Approximately 100 g (dry mass) of early summer leaves were obtained from each plant by removing all leaves from at least one branch of each of the specimens mentioned in Table 1. Voucher specimens of each sampled plant were deposited in the herbarium of the University of Potchefstroom.

The air-dried leaves of each plant were reduced to powder in a Retsch-mill and approximately 0.2 g samples of the thoroughly mixed leaf material were removed and accurately weighed. An internal standard of 0.02 μl of n-heptadecane (C_{17}) in 10 μl CCl_4 and a marker of 0.02 mg n-tetradetracontane (C_{44}) in 10 μl CCl_4 for determination of relative retention times, were added. C_{17} and C_{44} standards were chosen because none of the sampled specimens contained demonstrable amounts of these alkanes. After addition of 1 ml CCl_4 the mixture was shaken for 30 minutes at 45°. All the following steps were done at this temperature to ensure

TABLE 1

All voucher numbers refer to specimens collected by D. J. Botha and J. Coetzee. Alkane concentrations are expressed as percentage of the total with trace quantities not indicated.

No.	Species	Voucher No.	Locality	Carbon Chain Length																	Total alkanes mg/g dry mass							
				12	14	16	18	19	20	21	23	24	25	26	27	28	29	30	31	32		33	34	35	36	37		
1	<i>P. echinatus</i>	1603	Port Shepstone	0.2																						0.34		
2	<i>P. echinatus</i>	1886	Barnardsvlei		0.3	0.2										3.3	4.2	77.3	1.8	10.7	0.7	1.1				0.82		
3	<i>P. echinatus</i>	1887	Barnardsvlei														0.9	5.5	79.9	2.1	9.6	0.3	0.8				1.27	
4	<i>P. echinatus</i>	1937	Blydepoort		0.4	0.2										0.2	1.6	5.5	82.5	1.5	7.1	0.4	0.7				0.60	
5	<i>P. echinatus</i>	1938	Blydepoort													0.3	1.9	96.4	0.2	0.8						3.29		
6	<i>P. echinatus</i>	2141	Manepskop		0.5	0.2										2.4	4.3	78.1	2.6	9.6	0.5	1.1	0.2				0.31	
7	<i>P. echinatus</i>	2144	Manepskop		0.2											2.3	2.3	78.1	1.9	13.1	0.9	0.8					0.52	
8	<i>P. echinatus</i>	2146	Manepskop													0.2	4.5	5.7	6.1	2.8	8.2	0.7	0.6	0.3	0.2	0.2	0.44	
9	<i>P. echinatus</i>	2147	Manepskop		0.2	0.3										0.3	0.2	4.3	2.0	68.6	2.5	17.4	0.8	1.7	0.4	0.3	0.31	
10	<i>P. echinatus</i>	2149	Manepskop		0.2											2.9	12	72.8	1.3	18.3	0.9	1.8					0.56	
11	<i>P. echinatus</i>	2150	Manepskop		0.5	0.4	0.2									0.2	0.3	2.2	8.7	68.8	2.3	12.7	1.2	1.6	0.2	0.2	0.34	
12	<i>P. echinatus</i>	2151	Manepskop													0.3	0.2	4.0	1.8	72.1	3.1	14.9	1.0	1.5	0.3	0.3	0.39	
13	<i>P. echinatus</i>	2152	Manepskop		0.2											0.2	3.3	1.7	62.4	12.1	17.1	0.5	1.8	0.2	0.3		0.38	
14	<i>P. rostratus</i>	1642	Betty's Bay					0.2								0.8	1.3	3.2	41.0	29.5	14.5	6.8	1.9				0.18	
15	<i>P. rostratus</i>	1643	Betty's Bay		0.3	0.3	0.6	0.4								0.7	1.4	3.7	12.0	65.9	3.3	9.7	0.3	0.5			0.22	
16	<i>P. tricuspidatus</i>	1633	Tsitsikamma		0.2	0.2			0.2	0.2						0.4	0.6	3.4	3.9	67.9	3.1	17.1	1.7	0.9			0.26	
17	<i>P. tricuspidatus</i>	1635	Tsitsikamma													0.2	0.3	2.3	2.1	73.0	2.7	16.3	1.4	1.1			0.32	
18	<i>P. tricuspidatus</i>	1640	Betty's Bay				0.2			0.2						0.7	2.6	22.0	4.0	60.6	1.5	5.1	0.6	0.6			0.27	
19	<i>P. tricuspidatus</i>	1641	Betty's Bay		0.3	0.4										0.2	0.7	1.2	6.0	4.8	72.3	4.1	6.9	1.4	0.9		0.18	
20	<i>P. tricuspidatus</i>	1644	Betty's Bay		0.6	0.4		0.2	0.2							0.5	2.5	2.7	4.5	26.5	7.7	40.9	5.1	7.2	1.6	0.8		0.15
21	<i>P. tricuspidatus</i>	1711	Lamberts Bay		0.5			0.3	0.4	0.6						1.3	6.8	14.6	8.8	51.3	3.5	7.5	1.4	0.9			0.16	
22	<i>P. tricuspidatus</i>	1715	Lamberts Bay	0.6	2.3	1.3	0.2	0.3	0.2	0.3						0.9	2.7	11.0	13.9	54.3	1.0	9.6	0.4	1.0			0.11	
23	<i>P. tricuspidatus</i>	1718	Lamberts Bay		0.9	0.3	0.4	0.3	0.5	0.2	0.6					1.4	2.3	22.0	13.6	53.1		3.5	0.3	0.4			0.12	

complete solubility of the long chain alkanes. The mixture was subsequently passed through a 5×5 mm silica gel G column and the column eluted with 1 ml of CCl_4 . The total eluate was evaporated to dryness at room temperature under a stream of nitrogen. The residue was dissolved in $10 \mu\text{l}$ CCl_4 and $1 \mu\text{l}$ injected into the gas chromatograph. G.L.C. parameters: 3 mm $\times$ 2 m stainless steel column with 3 % SE-30 on Chromosorb 750, initial temp. 100° for 20 minutes, 3° rise per minute to 335° , final period 60 minutes. N_2 flowrate $10 \text{ cm}^3/\text{minute}$, F.I.D.

Peaks were identified by co-chromatography with even numbered carbon chain length n-alkane standards and odd chain lengths by the straight line plot of absolute retention times against carbon number. To allow for possible changes in chromatographic parameters between analyses, retention times relative to C_{44} were also plotted and used for identification. Peak areas were obtained by triangulation.

RESULTS

Table 1 illustrates the percentage of each n-alkane present in the samples. Only alkanes which are present at concentrations higher than 0.1 % are indicated, but even the lowest concentrations were taken into account in calculating the values, so that the values do not necessarily total 100 %.

All, except one of the sampled plants (*P. rostratus*, no. 14), have C_{29} as the largest constituent. In this and only one other additional plant (*P. tricuspidatus*, no. 20) is the total of all other alkanes more than 50 %. The domination of odd numbered carbon chain lengths as found in nearly all higher plants, is obvious. *P. rostratus* (no. 14) is highly unusual in that C_{28} is the dominant chain length. The absence of this obvious domination for the shorter chain lengths is probably to be found in the larger contribution of the "internal" alkanes to this fraction. These internal alkanes are known to have a "flat" distribution of chain lengths and to have a different composition from the "external" hydrocarbons (Herbin & Robins, 1969).

When the total alkane content of individual samples are plotted against the number of different chain length alkanes present in each sample (Figure 1), it is obvious that a negative correlation exists. The higher the amount of total alkanes, the lower the number of different chain lengths contributing to the total. The high total alkane content of samples are usually due to the presence of very large amounts (up to 96 %) of a single (C_{29}) chain length, with nearly all other fractions present in very low concentrations.

The disappearance of chain lengths other than the primary alkane may be due to the depletion of substrate materials during rapid synthesis of the waxes. This could lead to a rearrangement of existing alkanes to form the primary C_{29} alkane. If this is the case, it could be expected that only the "internal" hydrocarbons participate in this rearrangement, as the cuticular fraction cannot logically be expected to undergo such changes.

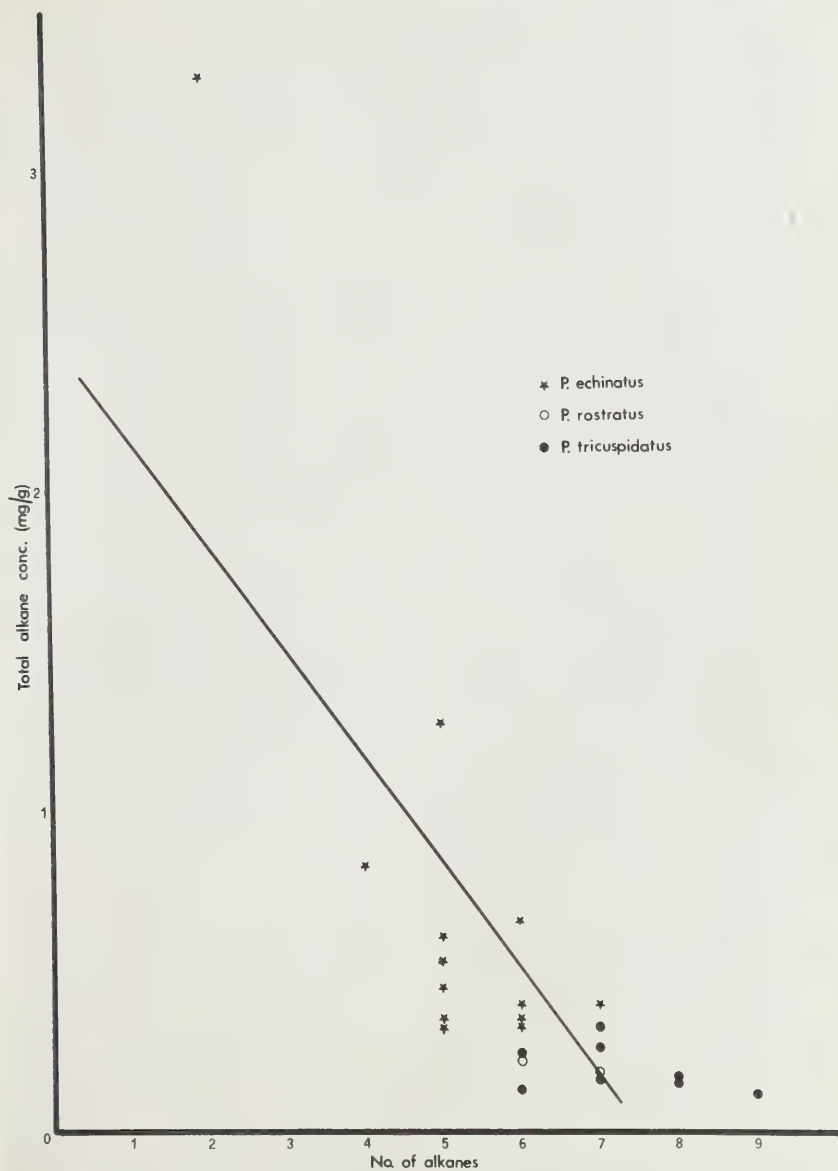


FIG. 1.

Negative correlation of -0.74 of total alkane content and number of alkanes found in each specimen. Linear regression line indicated.

The differences in alkane patterns observed in the studied samples cannot be ascribed primarily to environmental factors, as some of the plants with very different alkane compositions come from the same locality, as in case of the two specimens (nos. 4 and 5) of *P. echinatus* from Blydepoort and nos. 14 and 15 (*P. rostratus*) from Betty's Bay.

The alkanes of *Maytenus oleoides* and *Cassine eucleiformis* were also examined (results not given here) and the patterns proved to be very similar to those of *Pterocelastrus*. Variation of the patterns must therefore be due to genetic factors peculiar to each individual.

The data were processed with the aid of a number of numerical taxonomic computer programs, but all results showed that intraspecific variation is usually larger than the interspecific variation. No taxonomically valid deductions can thus be made from these results.

ACKNOWLEDGEMENTS

Thanks is due to Dr D. J. Botha for collection of plant material and the South African C.S.I.R. for a research grant.

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